1116-Symp
Experimental and Computational Approaches to Study Myofilament Structure-Function in Normal and Diseased Muscle
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We use a combination of site-directed mutagenesis, protein biochemistry, multi-scale mechanical analysis and computational modeling to study the regulation of normal muscle myofibril contraction and how this is altered in diseases of the sarcomere. This interdisciplinary approach allows us to do detailed structure-function analysis. Mutations in cardiac troponin C (cTnC) that increase Ca^{2+} binding affinity also increase its affinity for cardiac troponin I (cTnI) and when these cTnC mutants are exchanged into myofibrils or skinned trabeculae, they increase the magnitude and rate of force generation at sub-maximal, but not maximal levels of activation, and can slow the early phase of relaxation. In contrast, cTnC mutations that reduce Ca^{2+} binding affinity have either no effect or reduce interaction with cTnI, reduce the magnitude and rate of force generation at all levels of Ca^{2+} and speed relaxation. Molecular Dynamics (MD) simulations show positive correlation between Ca^{2+} binding affinity and stability of both 1) interaction of Ca^{2+} with coordinating side chains in site II and 2) the hydrophobic patch of cTnC. Together the data suggest that native cTnC may operate just at the edge of maximal effectiveness. Mutations in cTnI associated with hypertrophic cardiomyopathy increase its affinity for cTnC and also Ca^{2+} binding affinity of cTnI. Interestingly, they also blunt the ability of cTnI Ser 23/24 phosphorylation to reduce its affinity for cTnC and increase the rate of early phase relaxation. MD simulation studies of whole cTn suggest Ser 23/24 phosphorylation leads to the formation of the intra-subunit interaction between the N-terminus and the inhibitory peptide region of cTnI. We are studying how this, and other intra-molecular interactions may be affected by HCM associated mutations and the contraction and relaxation properties of cardiac muscle. HL65497, HL11197 (MR), 8P41GM103426 (AM).

1117-Symp
Effects of Transmural Region and Heart Failure on the Contractile Properties of Human Myocardium
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Heart failure is associated with pump dysfunction and remodeling but it is not yet known if the condition affects different transmural regions of the heart in the same way. We tested the hypotheses that tissue samples from the left ventricles of non-failing human hearts exhibit transmural heterogeneity of cellular level contractile properties, and that heart failure produces region-specific changes in contractile function. Chemically permeabilized samples were prepared from the sub-epicardial, mid-myocardial, and sub-endocardial regions of the left ventricle of non-failing (n=6) and failing (n=10) human hearts. Power, an in vitro index of systolic function, was ~20% higher in non-failing mid-myocardial samples (0.59 ± 0.06 μW mg^{-1}) than in samples from the sub-epicardium (p=0.021) and the sub-endocardium (p=0.015). Non-failing mid-myocardial samples also produced ~20% more isometric force (14.3 ± 1.33 kN m^{-2}) than samples from the sub-epicardium (p=0.008) and the sub-endocardium (p=0.026). Heart failure reduced power (p=0.009) and force (p=0.042) but affected mid-myocardial samples more than sub-epicardial and sub-endocardial tissue. Fibrosis increased with heart failure (p=0.021) and mid-myocardial tissue from failing hearts contained more collagen than the matching sub-epicardial ($p<0.001$) and sub-endocardial (p=0.043) samples. Myocardial power output was correlated with the relative content of actin (p=0.012), and the relative content (p=0.034) and phosphorylation (p=0.006) of myosin light chain-1. Passive force correlated with the phosphorylation of TnI at Ser23/24 (p=0.006) while shortening velocity increased in proportion with the phosphorylation of cMyBP-C at Ser282 (p=0.001). In conclusion, non-failing human hearts exhibit transmural heterogeneity of contractile properties. In failing organs, region-specific fibrosis produces the greatest contractile deficits in mid-myocardial tissue. Targeting collagen deposition and sarcomeric proteins in the mid-myocardium may be particularly effective therapies for heart failure.

1118-Symp
Mechanosignalling by Cytoskeletal Protein Kinases and their Disease Implications
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Many cellular processes involve the sensing and processing of mechanical force to trigger cellular responses. For such control mechanisms to act, the cell must contain sensors responding to changes in mechanical load. In muscle, increasing evidence points to a pivotal role of sensing mechanisms in the contractile machinery itself. Titin, the giant elastic ruler protein of sarcomeres, contains a catalytic kinase domain (TK) related to the myosin light-chain kinases (MLCK) family of intrasterically regulated protein kinases. The "MLCK" family is a diverse group of kinases whose unifying feature is their cytoskeletal association and exposure to mechanical stress. Scaffolding and catalytic activities of several of these kinases also communicate with protein turnover pathways. Recently, recessive human mutations in TK were identified that abrogate catalytic activity and scaffolding functions and cause severe early-onset myopathy, while two knock-in models of TK replicate either the loss of catalytic activity or scaffolding functions. Muscle atrophy by mechanical unloading was triggered in catalytically inactive TK mice by sciatic denervation. Significant changes in skeletal muscle fibre sizes under baseline and aggravated atrophy, with deregulated response of the autophagy-lysosomal system, replicate findings in human myopathy and support a non-redundant role in mechanically modulated muscle maintenance as well as under conditions of atrophy and hypertrophy in vivo. Similar to other MLCK-like kinases like DRAK2 and DAPK1, TK is linked to protein turnover regulation via the autophagy/lysosomal system, suggesting that MLCK-like kinases have common functions beyond contraction regulation. Modulation of ligand binding and catalytic activity by mechanical forces in cytoskeletal protein kinases may therefore be a common regulatory mechanism, which we are exploring using high-throughput single-molecule force spectroscopy measurements combined with single molecule fluorescence to understand low force modulation of ATP-hydrolysis and phosphotransfer mechanisms in other "MLCKs".

Platform: Membrane Physical Chemistry II

1119-Plat
Curved Lipid Bilayers: Structure, Dynamics, Phase Properties and Surface Electrostatics
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A growing body of experimental data suggests that at least some of membrane-anchored and membrane-associated proteins are capable of sensing the membrane curvature. Further, highly curved lipid bilayers and small vesicles are involved in such important cellular processes as membrane fusion, endo- and exocytosis, and tubules' formation. Finally, Golgi apparatus represents an example of highly curved lipid structure. While significance of membrane curvature in cellular regulatory processes is emerging, limited data exist on biophysical properties of highly curved lipid bilayers. Here we summarize results of differential scanning calorimetry and spin labeling EPR studies of unilamellar vesicles (SUV) with average diameter ranging from 200 to 30 nm. Analysis of DSC data at multiple scan rates revealed broadening and shifts of the main phase transition of DMPC from ca. 22.9 to 23.6 oC. This observation is consistent with bilayer compression and an increase in local order parameter reveled by EPR and oxygen accessibility measurements. To assess the surface electrostatics of lipid vesicles we employed EPR of a recently introduced phospholipid (IMTSL-PTE) bearing a pH-sensitive nitroxide covalently attached to the lipid head group (Biophys. J. 2013, 104: 106). The magnitude of the negative surface electrostatic potential, Ψ, for POPG increased from -137 to -167 mV upon decrease in the vesicle diameter from 107 to 31 nm even though zeta-potentials were identical. This effect could be again rationalized by increase in lipid packing upon increase in curvature for the bilayer in fluid phase. However, the effect vanished for the gel phase. We conclude that biologically relevant fluid bilayer phase allows for a larger variability in the lipid packing density in the lipid polar head group region than a more ordered gel phase. Supported by U.S. DOE Contract DE-FG02-02ER15354.

1120-Plat
Molecular Origins of the Ripple Phase
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The ripple phase of lipid bilayers is characterized by periodic ripples in one dimension. We explore the molecular origins of this peculiar phase. Since the ripple wavelength is of the order of the bilayer thickness, molecular

simulation can provide a detailed microscopic picture of lipid behavior in the ripple phase. In the first simulations of their kind, we study stacks of rippled bilayers to provide a more realistic molecular perspective that can be directly compared to X-ray diffraction and AFM experiments. We find that saddle-splay and tilt are key order parameters that characterize the long-range order observed in ripple phases. In addition, we elucidate the effects of lipid length, head group interaction, and bilayer hydration on ripple structure. The impact of lipid orientations in the ripple phase to biological processes such as membrane fusion is discussed.

1121-Plat

Influence of Detergent Properties on the Solubilization and Function of Membrane Proteins

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Detergent micelles are often used as a bilayer mimic for membrane proteins for functional assays and high-resolution structural studies, but selecting the appropriate detergent for the protein requires extensive time and resources. The long-term goal of this research is to rationally select a membrane mimic for a given membrane protein that stabilizes fold and native function based on matching physical properties of the detergent and protein. To determine the important micelle and membrane properties, lipoprotein signal peptidase A (LspA) from Chlamydia trachomatis is used as a model system. To screen detergents that support the native function, LspA was purified in over 30 detergent micelles with varying physical properties. LspA is soluble in zwitterionic detergents and solubility remains independent of detergent alkyl chain length, solubilizing in detergents with wide range of hydrophobic radius, 27-37Å. A detergent may maintain protein solubility without maintaining function or fold; therefore, the function of LspA in these detergents is currently being studied. To assess the activity of native membrane and solubilized LspA, a signal peptide containing the LspA cleavage consensus sequence and donor-quencher pair on each termini was designed. Preliminary HPLC and fluorescence data demonstrate a cleavage of the signal peptide upon addition of LspA in the native membrane and a comparison to different protein-detergent complexes will be presented. The results presented will identify solubilizing detergents, monitor enzymatic activity, and ultimately correlate the physical properties of the solubilizing detergent and protein structure and function.

1122-Plat

Quantitative Imaging of the Electrostatic Field of a Transmembrane Protein at Subnanometer Resolution by the use of Atomic Force Microscopy

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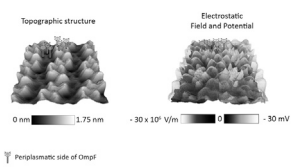
Elucidating the mechanisms by which proteins translocate small molecules and ions through transmembrane pores and channels is of great interest in biology, medicine and nanotechnology. These translocation mechanisms are mainly based on electrostatic interactions. However, the characterization of pore forming proteins in their native state lacks suitable methods that are capable of high-resolution imaging (≈1 nm) while simultaneously mapping physical and chemical properties. Here we report how force-distance (FD) curve based atomic force microscopy (AFM) imaging can be applied to image the native pore forming outer membrane protein F (OmpF) at sub-nanometer resolution and to quantify the electrostatic field and potential generated by the transmembrane pore. We further observe the electrostatic field and potential of the OmpF pore switching 'on' and 'off' in dependence of the electrolyte concentration. Because electrostatic field and potential select for charged molecules and ions and guide them to the transmembrane pore the insights are of fundamental importance to understand the pore function. These experimental results establish FD-based AFM as unique tool to image biological systems to sub-nanometer resolution and to quantify their electrostatic properties.



1123-Plat

Continuous Flow AFM Imaging Reveals Fluidity and Time Dependent Interactions of Antimicrobial Dendrimer with Model Lipid Membranes

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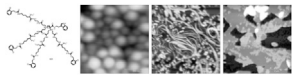
The alarming increase in multi-drug resistant bacteria worldwide is a major threat to modern life and there is thus an urgent need to address this issue by novel approaches in order to improve and tailor alternative drug formulations in the future. The nanoparticle studied here is a newly synthesized antimicrobial peptide-based dendrimer that acts by disrupting cell surfaces in a non-specific manner, thus rendering it less susceptible to resistance-forming mechanisms in bacteria.

The effects of bilayer fluidity and presence of domains have been studied using neutron reflection and an atomic force microscope setup optimized for continuous flow imaging, developed specifically for investigating interactions with fast kinetics.

Using these approaches we observed a requirement for membrane fluidity for dendrimers to induce high curvature and solubilize the membrane in a detergent-like manner. Domain coexistence led to a sequence of events initiated by the formation of a dense thread-like network and followed by membrane solubilisation via spherical aggregates from bilayer edges.

In contrast, for gel-phase membranes, the antimicrobial dendrimers adsorbed and caused areas of locally depressed regions - a mechanism resembling membrane interdigitation or membrane thinning.



1124-Plat

Exploring Continuum Models of Ion and Peptide Interactions with the Membrane

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Experimental and computational studies have shown that cellular membranes deform to stabilize the inclusion of transmembrane (TM) proteins harboring charge. Recent analysis suggests that membrane bending helps to expose charged and polar residues to the aqueous environment and polar head groups. We previously used elasticity theory to identify membrane distortions that minimize the insertion of charged TM peptides into the membrane. Here, we extend our work to consider the energetics of ion and small peptide penetration into the membrane. First, we show that our continuum method accurately reproduces energy profiles and membrane shapes generated from molecular simulations of bare ion permeation at a fraction of the computational cost. Next, we demonstrate that the apparent linear dependence of bare ion insertion energy on membrane thickness arises primarily from the elastic property of the membrane. Moreover, the continuum model readily provides free energy decompositions, still an obstacle for traditional molecular dynamics (MD). Finally, we show that the energetics of membrane deformation strongly depend on membrane patch size both for ions and peptides. This work therefore presents a novel, computationally efficient method for simulating the dynamics of small molecule and peptide interactions with the membrane bilayer.

1125-Plat

Histones and DNA Compete for Binding Phosphoinositides in Bilayers

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Recent discoveries on the presence and location of phosphoinositides in the eukaryotic cell nucleus prompted us to study the putative interaction of histones with these lipids in model membranes (liposomes). Turbidimetric studies revealed that a variety of histones and histone combinations (H1, H2AH2B, H3H4, octamers) caused a dose-dependent aggregation of phosphatidylcholine vesicles (LUV or SUV) containing negatively-charged phospholipids. 5 mol % PIP was enough to cause extensive aggregation under our conditions, while with PI at least 20 mol % was necessary to obtain a similar effect. Histone binding to GUV and vesicle aggregation was visualized by confocal microscopy. Histone did not cause vesicle aggregation in the presence of DNA, and the latter was able to disassemble the histone-vesicle aggregates. At DNA/H1 weight ratios 0.1-0.5 DNA- and PIP-bound H1 appear to coexist. Isothermal calorimetry studies revealed that the PIP-H1 association constant was one order of magnitude higher than that of PI-H1, but several orders of magnitude lower than that of H1-DNA. The results suggest that, in the cell nucleus, a complex interplay of histones, DNA and phosphoinositides may be taking place. The model system described here could help in analyzing these interactions.